REMARKS

Pending claims

Claims 25-35 and 67-79 are pending. No new matter is added by this response.

Formal Matters

Sequence Compliance

Transmitted herewith is a copy of the "Sequence Listing" in paper form for the above-identified patent application as required by 37 C.F.R. §1.821(c) and a copy of the Sequence ~~Listing in computer readable form as required by 37 C.F.R. §1.821(e). As required by 37 C.F.R.~~ §1.821(f), Applicant states that the content of the "Sequence Listing" in paper form and the computer readable form of the "Sequence Listing" are the same and, as required by 37 C.F.R. §1.821(g), also states that the submission includes no new matter.

Specification

Applicants have amended the title to more clearly indicate the claimed invention. Applicants have amended to specification to properly claim priority under 35 U.S.C. §119(e). With respect to the Examiner's indication that the Brief Description of Drawings for figures 4 and 7 do not refer to 4A-B and 7A-C, Applicants submit that the figures themselves to not refer to parts A-B or A-C, respectively. Accordingly, Applicants have not amended the Description for figures 4 and 7. All other amendments to the specification suggested by the Examiner, however, have been made. Applicants submit that the amendments do not introduce new matter.

Rejection of Claims 25-35 and 67-79 under 35 U.S.C. §112, first paragraph

Claims 25-35 and 67-79 are rejected under 35 U.S.C.§112, first paragraph for alleged lack of adequate written description and enablement.

The Examiner states, "the specification does not teach transferring the pseudo-islet aggregates into any patient, especially to treat diabetes mellitus." The Examiner also states,

"[t]he disclosure in the instant application is not adequate guidance, but is merely an invitation for the artisan to use the current invention as a starting point for further experimentation...the prophetic example does not teach the skilled artisan the optimal dosage, duration, and mode of administration of the pseudo-islet like aggregates...[t]he skilled artisan must resort to trial and error experimentation to determine the optimal dosage, duration, and mode of administration of the pseudo-islet like aggregates".

The Examiner also asserts that [t]he specification does not teach that the transplanted pseudo-islet like aggregates act like healthy islet cells...there are no methods of working examples that indicate the pseudo-islet like aggregates would have any effect on blood glucose insulin, which is required for diabetes treatment."

The Examiner also asserts that "patients may suffer one of two types of graft or transplant rejections, host-versus-graft rejection or graft-versus-host rejection...[s]ince claim 25 recites that the patient does not serve as the donor for the nestin-positive pancreatic stem cells, the skilled artisan cannot predict that the differentiated pseudo-islet like aggregates can be successfully immunologically transplanted into the recipient patient...the specification of the instant application does not disclose the identity of the nestin-positive pancreatic stem cell donor...the cells could be from another human, a pig, monkey rat, etc. and therefore possibly cause host-versus-graft rejection or GvHR in the recipient patient.".

Applicants respectfully disagree.

Enablement

To meet the enablement requirement, Applicants must provide sufficient teaching in the specification to enable one of ordinary skill in the art to make and use the present invention without undue experimentation. Applicants submit that the specification teaches how to isolate nestin-positive pancreatic stem cells from a donor (page 42-43), how to expand the stem cells to produce progenitor cells (page 43-44), how to transfer the pseudo-islet like progenitors into a patient (Example 8, 9, and 10, and pages 38-39). Thus, Applicants assert that the claims are

enabled by the specification, as one of skill in the art could reproduce the invention with only routine experimentation.

The Examiner asserts that the specification is not enabling because it does not teach transferring the pseudo-islet like aggregates into a patient to treat diabetes mellitus. Applicants disagree. The specification teaches on page 33-34 that pseudo-islet like aggregates may be administered to a patient in need thereof to treat diabetes. The specification specifically addresses the Examiner's concern regarding host vs. graft (and vice versa) rejection, teaching that the pseudo-islet like aggregates may be adminstered to a patient along with a blocking antibody such as GAD65. The Examiner asserts further that the specification does not teach the skilled artisan the optimal dosate, duration, and mode of adminstration of the pseudo-like aggregates. Applicants submit that the specification teaches on page 38 that "[a] preferred method [of administration] is endoscopic retrograde injection", and that islet progenitor cells are to be administered at a concentration of "in the range of 10^5 - 10^8 cells per kg body weight, preferably in the range of 10^6-10^7 cells per kg body weight" (page 39). Thus, Applicants submit that the specification provides sufficient teaching to enable one of skill in the art to practice the invention without undue experimentation.

It is well established that post-filing documentation or publications may be used to demonstrate that Applicants disclosure was enabling at the time of filing (*See, e.g., In re Brana*, 51 F.3d 1560 (Fed. Cir. 1995)). Accordingly, Applicants submit herewith, Exhibits A and B which demonstrate that the methods disclosed by Applicants in the present application were sufficient to enable one of skill in the art to practice the claimed method of treating a patient with diabetes mellitus as of the filing date.

Applicants assert that there is post-filing date literature teaching treatment of diabetic SCID mice by implantation of human derived nestin positive stem cell aggregates that resemble islets (NPAs). (see abstract, Exhibit A; From meeting "Pancreatic Development, Proliferation, and Stem Cells", October 18-19, 2001, Bethesda M.D.). A second post-filing date abstract (Exhibit B; From U.C. Davis/VSTP Retreat (Schedule of events attached), August 26, 2002) discloses that human NPAs implanted into streptozotocin induced diabetic mice, (blood glucose

concentration >300 mg/dl) were able to maintain essentially normal glucose concentrations, without exogenous insulin, for more than 70 days, and exhibited essentially normal glucose tolerance tests. Exhibit B teaches implantation of 2-5 x 10^5 aggregates per mouse.

These abstracts demonstrate the actual reduction to practice of the invention of the instant application, as claimed in independent claims 25 and 67. "Pseudo-islet like aggregates" are defined in the application at page 13, lines 17-21. Applicants submit that one of skill in the art would accept that the nestin positive stem cell aggregates described in Exhibit A and B are equivalent to the "pseudo-islet like" aggregates of the instant application. That is, the nestin positive stem cell aggregates of Exhibits A and B are "artificial aggregates of insulin-secreting cells which resemble in form and function the islets of Langerhans of the pancreas", as described in the instant application at page 13, lines 17-21; page 29, lines 17-24; and Examples 2 and 3.

The data presented in these abstracts demonstrate the treatment of diabetes mellitus by the transfer of pseudo-islet aggregates into a patient. Exhibit B teaches that between 2-5 x10^5 aggregates were introduced to each mouse; a dosage consistent with that taught by the present specification.

Both Exhibit A and B demonstrate that, in contrast to the Examiner's assertion, the transplanted pseudo-islet like aggregates act like healthy islet cells and effect blood glucose insulin, resulting in diabetes treatment.

Both Exhibit A and B demonstrates the successful implantation of human nestin-positive cell aggregates into mice. That is, data presented in Exhibit A and B demonstrate that diabetes can be treated, without the occurrence of transplant rejection or graft rejection, even if the patient is not the donor for the nestin-positive aggregates. Thus, in view of the teachings of the specification, in further view of these abstracts, one of skill in the art would have no reason to predict that graft or transplant rejection would occur if a human patient were transplanted with pseudo-like aggregates derived from any of the following: another human, a pig, monkey rat, etc. Thus, Applicants submit that where the methods described in Exhibits A and B essentially mirror the teaching of the present specification, one of skill in the art would have been able to

reproduce the claimed invention as of the filing date, without undue experimentation. Although, the studies performed in Exhibits A and B were conducted in an art recognized animal model of diabetes, Applicants submit that *In re Brana* also provides that such *in vivo* animal testing is sufficient to meet the patentability requirements for an invention relating to the treatment of humans. *Id.*

With respect to the Examiner's assertion that Edlund (Nature Rev. Genet. 3: 524 (2000)) teaches that since nestin is not expressed in pancreatic epithelial cells at any stage of development, nestin is an inappropriate marker for pancreatic cell types. Applicants submit that both the Examples provided in the present specification and the teachings provided in Exhibits A and B demonstrate that nestin-positive stem cells may be differentiated to express pancreatic islet markers and to modulate glucose levels *in vivo*. Moreover, a brief search of the MEDLINE database identified a number of references indicating that nestin-positive stem cells in the developing pancreas are able to differentiate into insulin producing cells (See, e.g., Hunziker and Stein, 2000, *BBRC* 271:116; Blyszczuk et al., 2003, *PNAS* 100: 998; Abraham et al., 2002, *Endocrinology* 143:3152; Lechner et al., 2002, *BBRC* 293:670). Thus, Applicants submit that it appears to be Edlund who is contradictory to the state of the art, and not the teaching of the present invention.

With respect to the Examiner's assertion that the specification does not permit one of skill in the art to predict the activity and immunologic effects of the pseudo-islet like aggregates once administered to the patient, Applicants respectfully submit that the predictability of each and every possible embodiment of the invention is not required under the law.

Applicants submit that the statute does not require absolute predictability of the activity of all embodiments. In *In re Wands* (858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988)) it could not have been predicted *a priori* which hybridomas of those made would be active. The hybridomas needed to be screened. Indeed, absolute predictability of the activity of embodiments which may be embraced within the claims is not a requirement of the statute. The decision in *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that every embodiment need not be disclosed, even in an unpredictable art, and clearly permits the presence

of a screening step to identify those embodiments which possess the desired activity. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that would predict the outcome of an experiment (or reaction) "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts."

The Federal Circuit has also stated that

> . . . we do *not* imply that patent applicants in art areas currently denominated as 'unpredictable' must never be allowed generic claims encompassing more than the particular species disclosed in their specification. It is well settled that patent applicants are not required to disclose every species encompassed by their claims, even in an unpredictable art.

In re Vaeck, 947 F.2d 731, 20 U.S.P.Q.2d 1438, 1445 (Fed. Cir. 1991), citing *In re Angstadt*, 537 F.2d 498, 502-3, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976), emphasis original.

In addition, the Federal Circuit has stated that "[w]hat is patented is not restricted to the examples, but is defined by the words in the claims if those claims are supported by the specification in the manner required by 35 U.S.C. §112." (*Specialty Composites v. Cabot Corp.*, 845 F.2d 981, 6 U.S.P.Q.2d 1601 (Fed. Cir. 1988), at 1604).

The Federal Circuit has also held that claims may encompass some inoperative species, so long as the number of inoperative species does not become significant and force one of ordinary skill into undue experimentation in order to practice the invention (*Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 224 U.S.P.Q. 409 (Fed. Cir. 1984)).

It is therefore well settled that under 35 U.S.C. § 112, first paragraph, absolute predictability is not required, the claims may encompass some inoperative species, and that some experimentation is permitted. This implicitly permits the absence of absolute predictability of each claimed embodiment.

Applicants thus submit that the claims are fully enabled as of their filing date, and that one of skill in the art would be able to reproduce the claimed invention without undue experimentation. Applicants accordingly request that the rejection be reconsidered and withdrawn.

Rejection of Claims 25-35, 72 and 79 Under 35 U.S.C. §112, Second Paragraph

Claims 25-35, 72 and 79 are rejected under 35 U.S.C. 112, Second Paragraph for alleged indefiniteness.

The Examiner states that the acronyms "EGF", "bFGF-2", "KGF", "HGF/SF", "GLP-1", "IDX-1", "TGF-B", "FK-506" and "GAD65" renders the claims vague and indefinite. Abbreviations should be spelled out in all independent claims for clarity."

Claims 25-35, 72 and 79 have been amended to spell out the abbreviations.

The Examiner also states that claims 25-35 are indefinite because the claims do not have a step that clearly relates back to the preamble...there is no step indicating that transferring the pseudo-islet aggregates into the patient treates diabetes mellitus.

Applicants have amended claims 25-35 to a proper form.

In view of the above, Applicant respectfully requests withdrawal of the 35 U.S.C. §112, first paragraph rejection of claims 25-35 and 67-79.

Rejection of Claims 25, 28-30 and 35 for Obviousness-type Double Patenting

Claims 25, 28-30 and 35 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 13 and 15-18 of copending Application No. 09/731,261 and claims 19 and 24-27 of copending Application No. 09/963,875. The Examiner states that although the conflicting claims are not identical, they are not patentably distinct from each other.

In response to this rejection, Applicants submit that they will submit a terminal disclaimer

to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 09/731,261 and 09/963,875 applications, upon notification of allowable claims in the present application.

Rejection of Claims 67-68, 72-74 and 79 for Obviousness-type Double Patenting

Claims 67-68, 72-74, and 79 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 13-18 of copending Application No. 09/731,261 and claims 19 and 234-27 of copending Application No. 09/963,875. The Examiner states that although the conflicting claims are not identical, they are not patentably distinct from each other.

In response to this rejection, Applicants submit that they will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 09/731,261 and 09/963,875 applications, upon notification of allowable claims in the present application.

CONCLUSION

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

Respectfully submitted,

Date: April 4, 2003

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Marked-up version of amendments

In the Claims

25. (Amended) A method of treating a patient with diabetes mellitus, comprising the steps of:

(a) isolating a nestin-positive pancreatic stem cell from a pancreatic islet of a donor;

(b) expanding the stem cell to produce a progenitor cell;

(c) differentiating the progenitor cell in culture to form pseudo-islet like aggregates; and

(d) transferring the pseudo-islet like aggregates into the patient,

wherein the patient does not serve as the donor for said stem cells of step (a), and wherein said transferring step (d) treats diabetes mellitus.

26.(Amended) The method of claim 25, wherein the patient is a human and the donor for said stem cells of step (a) is a non-human mammal.

28. (Amended) The method of claim 25, wherein the step of expanding is performed in the presence of an agent selected from the group consisting of Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor-2 (bFGF-2), high glucose, Keratinocyte Growth Factor (KGF), Hepatocyte Growth Factor/Scatter Factor (HGF/SF), Glucagon-like-Peptide-1 (GLP-1), exendin-4, Islet/Duodenum Homeobox-1 (IDX-1), a nucleic acid molecule encoding Islet/Duodenum Homeobox-1 (IDX-1), betacellulin, activin A, Transforming Growth Factor-β (TGF-β), and combinations thereof.

68. (Amended) The method of claim 67, wherein the mammal serves as the donor for said stem cells of step (a).

69. (Amended) The method of claim 67, wherein the mammal does not serve as the donor for said stem cells of step (a).

72. The method of claim 67, wherein the step of expanding is performed in the presence of an agent selected from the group consisting of Epidermal Growth Factor (EGF), basic Fibroblast Growth Factor-2 (bFGF-2), high glucose, Keratinocyte Growth Factor (KGF), Hepatocyte Growth Factor/Scatter Factor (HGF/SF), Glucagon-like-Peptide-1 (GLP-1), exendin-4, Islet/Duodenum Homeobox-1 (IDX-1), a nucleic acid molecule encoding Islet/Duodenum Homeobox-1 (IDX-1), betacellulin, activin A, Transforming Growth Factor-β (TGF-β), and combinations thereof.

In the Specification

Please **replace** the paragraphs at p. 19 describing Figure 8 with the following.

Figure 8A-E depicts expression of the neural stem cell-specific marker nestin in a distinct cell population within pancreatic islets as determined by immunocytochemistry or RT-PCR.

Please **replace** the paragraphs at p. 19 describing Figure 9 with the following.

Figure 9A-C depicts characterization of nestin in stem cells isolated from the pancreas by immunocytochemistry and RT-PCR.

Please **replace** the paragraphs at p. 19 describing Figure 10 with the following.

Figure 10A-D depicts expression of homeodomain protein IDX-1 and proglucagon in human islet-like clusters derived from nestin-positive islet progenitor cells (NIPs).

Please **replace** the paragraphs at p. 19 describing Figure 11 with the following.

Figure 11A-C demonstrates localization of nestin-positive cells to localized regions of the ducts of the rat pancreas.

Please **replace** the paragraphs at p. 19 describing Figure 15 with the following.

Figure 15A-C depicts expression of neuroendocrine, exocrine pancreatic and hepatic markers in human NIP cultures containing stem cells.

Please **replace** the paragraph at page 18, lines 21 through 23, with the following replacement paragraph:

--Figure 2 shows the result of RT-PCR performed using mRNA obtained from 50 rat islets. Forward and reverse primers are indicated. The depicted primer sequences are: forward primer GCGGGGCGGTGCGTGACTAC (SEQ ID No: 3) and reverse primer GGGTGGTGAGGGTTGAGGTTTGTG (SEQ ID No: 55). The single band of 834 bp was sequenced and identified substantially as the sequence for nestin.--

Please replace the paragraph at page 19, line 3, with the following replacement paragraph:

--Figure 7 [is] depicts the nestin amino acid (SEQ ID No: 2) and nucleotide (SEQ ID No:1) sequences.--

Please **replace** the paragraph at pages 22, lines 19 through 28 continuing to page 23, lines 1 through 28 and page 24, lines 1 through 21, with the following replacement paragraph:

--RT-PCR and Southern blot analysis are performed according to the following methods. Total cellular RNA prepared from rat or human islets is reverse transcribed and amplified by PCR for about 35 cycles depending on the desired degree of amplification, as described previously (Daniel, et al., 1998, Endocrinology, 139:3721-3729). Oligonucleotides used as primers or amplimers for the PCR and as probes for subsequent Southern blot hybridization are:

Rat nestin:	Forward, 5'gcggggcggtgcgtgactac3' (SEQ ID NO: 3);
	Reverse, 5'aggcaaggggaagagaaggatgt3'(SEQ ID NO: 4);
	Hybridization, 5'aagctgaagccgaatttccttgggataccagagga3' (SEQ ID NO: 5).
Rat keratin 19:	Forward, 5'acagccagtacttcaagacc3'(SEQ ID NO: 6);
	Reverse, 5'ctgtgtcagcacgcacgtta3'(SEQ ID NO: 7);
	Hybridization, 5'tggattccacaccaggcattgaccatgcca3' (SEQ ID NO: 8).
Rat NCAM:	Forward, 5'cagcgttggagagtccaaat3'(SEQ ID NO: 9);
	Reverse, 5'ttaaactcctgtggggttgg3'(SEQ ID NO: 10);
	Hybridization, 5'aaaccagcagcggatctcagtggtgtggaacgatgat3'(SEQ ID NO: 11).
Rat IDX-1	Forward, 5'atcactggagcagggaagt3'(SEQ ID NO: 12)
	Reverse, 5'gctactacgtttcttatct3' (SEQ ID NO: 13)
	Hybridization, 5'gcgtggaaaagccagtggg3'(SEQ ID NO: 14)

Human nestin: Forward, 5'agaggggaattcctggag3'; (SEQ ID NO: 15)
Reverse, 5'ctgaggaccaggactctcta3'; (SEQ ID NO: 16)
Hybridization, 5'tatgaacgggctggagcagtctgaggaaagt3'.(SEQ ID NO: 17)

Human keratin: Forward, 5'cttttcgcgcgcccagcatt3';(SEQ ID NO: 18)
Reverse, 5'gatcttcctgtccctcgagc3';(SEQ ID NO: 19)
Hybridization, 5'aaccatgaggaggaaatcagtacgctgagg3'.(SEQ ID NO: 20)

Human glucagon: Forward, 5'atctggactccaggcgtgcc3';(SEQ ID NO: 21)
Reverse, 5'agcaatgaattccttggcag3'; (SEQ ID NO: 22)
Hybridization, 5'cacgatgaatttgagagacatgctgaaggg3'; (SEQ ID NO: 23)

Human E-Cadherin Forward, 5' agaacagcacgtacacagcc 3'(SEQ ID NO: 24)
Reverse, 5'cctccgaagaaacagcaaga 3'(SEQ ID NO: 25)
Hybridization, 5' tctcccttcacagcagaactaacacacggg 3'(SEQ ID NO: 26)

Human transthyretin Forward, 5' gcagtcctgccatcaatgtg 3'(SEQ ID NO: 27)
Reverse, 5' gttggctgtgaataccacct 3'(SEQ ID NO: 28)
Hybridization, 5' ctggagagctgcatgggctcacaactgagg 3' (SEQ ID NO: 29)

Human Pancreatic Amylase Forward, 5'gactttccagcagtcccata 3' (SEQ ID NO: 30)
Reverse, 5' gtttacttcctgcagggaac 3'(SEQ ID NO: 31)
Hybridization, 5' ttgcactggagaaggattacgtggcgttcta 3'(SEQ ID NO: 32)

Human procarboxypeptidase Forward, 5' tgaaggcgagaaggtgttcc 3' (SEQ ID NO: 33)
Reverse, 5' ttcgagatacaggcagatat 3'(SEQ ID NO: 34)
Hybridization, 5' agttagacttttatgtcctgcctgtgctca 3'(SEQ ID NO: 35)

Human Synaptophysin Forward, 5' cttcaggctgcaccaagtgt 3'(SEQ ID NO: 36)
Reverse, 5' gttgaccatagtcaggctgg 3'(SEQ ID NO: 37)
Hybridization, 5' gtcagatgtgaagatggccacagacccaga 3'(SEQ ID NO: 38)

Human Hepatocyte Growth Factor (HGF)
Forward, 5' gcatcaaatgtcagccctgg 3'(SEQ ID NO: 39)
Reverse, 5' caacgctgacatggaattcc 3'(SEQ ID NO: 40)
Hybridization, 5' tcgaggtctcatggatcatacagaatcagg 3'(SEQ ID NO: 41)

Human cMET (HGF-receptor)Forward, 5' caatgtgagatgtctccagc 3'(SEQ ID NO: 42)

Reverse, 5' ccttgtagattgcaggcaga 3'(SEQ ID NO: 43)

Hybridization, 5' ggactcccatccagtgtctccagaagtgat 3'(SEQ ID NO: 44)

Human XBP-1 Forward, 5'gagtagcagctcagactgcc 3'(SEQ ID NO: 45)

Reverse, 5' gtagacctctgggagctcct 3'(SEQ ID NO: 46)

Hybridization, 5' cgcagcactcagactacgtgcacctctgca 3'(SEQ ID NO: 47)

Human Glut-2 Forward, 5' gcagctgctcaactaatcac 3'(SEQ ID NO: 48)

Reverse, 5' tcagcagcacaagtcccact 3'(SEQ ID NO: 49)

Hybridization, 5' acgggcattcttattagtcagattattggt 3'(SEQ ID NO: 50)

Human Insulin Forward, 5' aggcttcttctacaca3'(SEQ ID NO: 51)

Reverse, 5' caggctgcctgcacca 3'(SEQ ID NO: 52)

Hybridization, 5' aggcagaggacctgca 3'(SEQ ID NO: 53).--

Please **replace** the paragraph at pages 50, lines 3 through 28 with the following replacement paragraph:

--Total cellular RNA prepared from rat or human islets was reverse transcribed and amplified by PCR for 35 cycles as described previously (Daniel et al., 1998, Endocrinology, 139:3721-3729). The oligonucleotides used as primers or amplimers for the PCR and as probes for subsequent Southern blot hybridization are:

Rat nestin: Forward, 5'gcggggcggtgcgtgactac3' (SEQ ID NO: 3);

Reverse, 5'aggcaaggggaagagaaggatgt3'(SEQ ID NO: 4);

Hybridization, 5'aagctgaagccgaatttccttgggataccagagga3' (SEQ ID NO: 5).

Rat keratin 19: Forward, 5'acagccagtacttcaagacc3'(SEQ ID NO: 6);

Reverse, 5'ctgtgtcagcacgcacgtta3'(SEQ ID NO: 7);

Hybridization, 5'tggattccacaccaggcattgaccatgcca3' (SEQ ID NO: 8).

Rat NCAM: Forward, 5'cagcgttggagagtccaaat3'(SEQ ID NO: 9);

Reverse, 5'ttaaactcctgtggggttgg3'(SEQ ID NO: 10);

Hybridization, 5'aaaccagcagcggatctcagtggtgtggaacgatgat3'(SEQ ID NO: 11).

Rat IDX-1 Forward, 5'atcactggagcagggaagt3'(SEQ ID NO: 12)

Reverse, 5'gctactacgtttcttatct3' (SEQ ID NO: 13)

	Hybridization, 5'gcgtggaaaagccagtggg3'(SEQ ID NO: 14)
Human nestin:	Forward, 5'agaggggaattcctggag3'; (SEQ ID NO: 15)
	Reverse, 5'ctgaggaccaggactctcta3'; (SEQ ID NO: 16)
	Hybridization, 5'tatgaacgggctggagcagtctgaggaaagt3'.(SEQ ID NO: 17)
Human keratin:	Forward, 5'cttttcgcgcgcccagcatt3';(SEQ ID NO: 18)
	Reverse, 5'gatcttcctgtccctcgagc3';(SEQ ID NO: 19)
	Hybridization, 5'aaccatgaggaggaaatcagtacgctgagg3'.(SEQ ID NO: 20)
Human glucagon:	Forward, 5'atctggactccaggcgtgcc3';(SEQ ID NO: 21)
	Reverse, 5'agcaatgaattccttggcag3'; (SEQ ID NO: 22)
	Hybridization, 5'cacgatgaatttgagagacatgctgaaggg3'; (SEQ ID NO: 23).--

Please **replace** the paragraph on page 53, lines 22 through 28 continuing to page 54, lines 1 through 3 with the following replacement paragraph:

--Insulin and glucagon concentrations in culture media were determined by ultra sensitive radioimmunoassay kits purchased from Linco Research Inc. and DPC Inc., respectively. The antisera supplied in the respective kits are guinea pig anti-human insulin and rabbit anti-human glucagon. GLP-1 secretion was measured with an anti-human GLP-1(7-36)amide rabbit polyclonal antiserum raised by immunization of a rabbit with a synthetic peptide CFIAWLVKGR (SEQ ID NO: 54) amide conjugated to keyhole limpet hemocyanin. The antiserum is highly specific for the detection of GLP-1(7-36)amide and only weakly detects proglucagon. The sensitivity levels for these assays are 6 pg/mL, 13 pg/mL and 10.2 pg/mL, respectively.--